Fruit Quality of Transgenic ‘Meeker’ Red Raspberry with Resistance to *Raspberry Bushy Dwarf Virus*

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Keywords: RBDV resistance, fruit quality, red raspberry, transgenic, volatile, SBSE

Abstract

Raspberry bushy dwarf virus **(RBDV) causes significant reduction in yield and crumbly fruit in raspberries, blackberries and raspberry-blackberry hybrids; the only means of control is through development of resistant cultivars. Genetic modifications have been made to ‘Meeker’ red raspberries in an effort to develop resistance to RBDV while maintaining the desirable fruit characteristics of ‘Meeker’ including sweetness, aroma profile and machine harvestability. Transgenic and wild-type ‘Meeker’ plants were grown in Oregon and Washington to compare the fruit quality under different climatic conditions over several years and to evaluate field resistance to RBDV. After six field seasons, only one of the transgenic lines was still completely free of RBDV under extreme disease pressure. The wild-type ‘Meeker’ plants in the same plots were 100% infected (202/202) after three field seasons. This transgenic line along with four other lines that were less than 100% infected after six field seasons was not infected with RBDV by grafting after three attempts. Fruit quality indicators such as °Brix, titratable acidity and sugar and organic acid profiles were performed on fruit from wild-type and five transgenic lines of ‘Meeker’ raspberries grown in Oregon and Washington for the years 2004 and 2005. Significant differences ($p<0.05$) were found between the years for all tests performed. °Brix and titratable acidity were also significantly affected by locations with berries grown in Washington having higher acid and lower °Brix than Oregon. The RBDV-resistant transgenic lines were similar to the wild-type ‘Meeker’ red raspberry. Experimental results showed that fruit quality is affected by season and growing environments. Additionally, the five transgenic lines behaved similarly to the wild-type ‘Meeker’ raspberry in yield. These results demonstrated that the transgenic RBDV-resistant lines of ‘Meeker’ could serve as a replacement for the wild-type ‘Meeker’ red raspberry provided that the fruit was accepted in the marketplace.**

INTRODUCTION

Raspberry production in the Pacific Northwest, Oregon and Washington in the USA and southwestern British Columbia in Canada, increased dramatically during the 1990s and the acreage has stabilized the past few years. Since the early 1980s, ‘Meeker’ has become the cultivar of choice for most growers because it outyields ‘Willamette’, has an intermediate level of resistance to root rot and produces a higher quality berry for the valuable fresh and whole frozen berry markets. With the change in cultivars and increased planting density there has been a rapid increase in the incidence of *Raspberry bushy dwarf virus* (RBDV), since ‘Meeker’ is susceptible to the virus while ‘Willamette’ is immune (Daubeny et al., 1982).

Flavors in raspberry are mainly formed during a brief ripening period and are influenced by numerous factors [19–21]. These factors include internal genetic makeup, and external agronomical factors, such as climate and soil type, as well as the ripeness and handling of the fruit [1, 22–25]. Sugars and organic acids are strongly affected by climate variations. Volatile compounds are generated through numerous pathways and their final concentrations are affected by environmental factors to various degrees due to effects on precursors and enzymatic activity within the fruit [25, 26].

'Meeker' red raspberry has been developed that contain several different RBDV constructs including: nontranslatable RNA, coat protein and three different mutated movement protein constructs (Martin et al., 2001). The objective of this study was to determine if the volatile composition of RBDV-resistant lines varies from wild-type 'Meeker' raspberry.

MATERIALS AND METHODS

Fruit Samples

Ripe wild type and transgenic 'Meeker' raspberries were harvested from Lynden, Washington in July 2004 and 2005 and Aurora, OR in June 2004 and 2005, when the fruits were fully ripe. The fruit were chilled and transported to the laboratory where they were individually quick frozen (IQF) and stored at -38°C until analyses were performed.

°Brix and Titratable Acidity

One hundred grams of red raspberries were thawed at room temperature for 3 hr. The juice collected from the thawing process was used to analyze °Brix using a refractometer. The berries were blended with 50 g of boiling distilled water at high speed for 30 sec then placed in a boiling water bath for 5 min to deactivate enzymatic activity. The berry mixture was then centrifuged at 2000 rpm for 20 min. and the supernatant was collected for analysis. Seven ml of the juice was combined with 50 ml of CO_2 free water and then titrated with 0.1N NaOH solution to an end point of pH 8.1. The results were reported as percent (%) of citric acid.

Extraction of Volatile Compounds

One hundred fifty grams of red raspberries were thawed for 3 hr then blended with 1% $CaCl_2$ and 10% NaCl in a commercial blender for 30 sec. The calcium chloride was added to inhibit enzyme activity and the sodium chloride was added to increase sensitivity [28]. The mixture was centrifuged at 2000 rpm for 20 min and the supernatant collected.

A Stir Bar Sorptive Extraction (SBSE) stir bar (1 cm long, 0.32 mm O.D. 0.5 mm film thickness) with a polydimethylsiloxane (PDMS) phase was used for the extraction of volatile compounds. The stir bar was cleaned with 80% acetonitrile in methanol overnight, allowed to air dry for 1 hr, then conditioned for 45 min at 300°C with 50 ml/min nitrogen flow. Ten grams of juice samples were weighed into 20 ml clear glass vials with polytetrafluorethylene septum caps and 10 µl internal standard mixture in methanol was added. The juice was extracted at room temperature for 1 hr at 1000 rpm. All samples were analyzed in triplicate.

Gas Chromatography-Mass Spectroscopy (MS)

The analysis of volatile compounds was performed with an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel MPS-2 multipurpose TDU autosample. The analytes were thermally desorbed at the TDU in splitless mode, ramping from 35ºC to 300ºC at a rate of 700ºC/min and held at the final temperature for 3 min. The CIS-4 was cooled to -80°C with liquid nitrogen during the sample injection, then heated at 10°C/sec to 250°C for 3 min. Solvent vent mode was used during the injection with a split vent purge flow of 50 ml/min beginning at 3 min. The helium column flow was 2.0 ml/min. Separation was achieved using a ZB-FFAP column (30 cm x 0.32 mm I.D., 0.5 µm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40°C for 2 min, then to 180°C at a rate of 6°C/min, then to 240°C at a rate of 4°C/min and held at the final temperature for 20 min. Standard EI mode was used at 70 eV. The total mass ion chromatogram was obtained from 35 to 350 amu. System software control and data management/analysis were performed through Enhanced ChemStation Software (Agilent Techonologies, Inc.).

RESULTS AND DISCUSSION

Wild-type and transgenic 'Meeker' raspberries grown in Lynden, Washington and Aurora, Oregon were studied for two years. The average high temperatures in Lynden, Washington in June and July (20°C and 23°C, respectively) are slightly cooler than those in Aurora, Oregon (23°C and 27°C, respectively), while the temperatures at night are similar (9°C and 11°C) in Washington and (9°C and 12°C) in Oregon. The average rainfall for June and July in Washington (66 mm and 50.8 mm, respectively) is considerably higher than in Oregon (44.4 mm and 18.5 mm, respectively). These differences allow for the comparison of transgenic lines and wild-type 'Meeker' red raspberry grown under different environmental conditions.

The °Brix, titratable acidity and °Brix/TA ratio for the wild-type and transgenic 'Meeker' raspberries grown in Washington and Oregon during 2004 and 2005 are presented in Table 1. Year to year and site to site variations were observed. For Washington and Oregon sites, fruit from 2004 had higher °Brix and lower acidity than fruits from 2005; although, the degree of difference was dependent on the site and year. In both years the raspberries grown in Washington had slightly lower °Brix and higher titratable acidity than the raspberries grown in Oregon. On average, raspberries grown in Oregon in 2005 had 30% higher °Brix and 20% lower titratable acidity than those grown in Washington. These differences correlated well with the climatic differences, Oregon had a higher average temperature than Washington. Climate variations are known to affect the flavor of fruits during the growing and ripening seasons, warmer and drier weather generally produce fruit with higher sugar and lower acid contents [1].

Sugar and acid contents are also affected by fruit maturity. Sugar concentration typically increases drastically during fruit ripening and continues through the overripe stage [21, 30]. Acid concentration typically increases early in fruit development, but decreases as the fruit ripens [1]. The higher °Brix number always corresponded with lower titratable acidity and was consistent across sites and years. Sugar, acid and °Brix/TA ratio are often used as maturity indicator and is particularly important for flavor perception [30].

During the 2004 and 2005 growing seasons, the °Brix and titratable acidity of all transgenic lines were not different from wild-type within a site and growing season. The °Brix values for all lines grown in Washington in 2005 were within the range reported previously[29].

A total of 30 volatile compounds were compared for all transgenic lines and the wild-type 'Meeker' grown in Oregon and Washington in 2004 and 2005. These compounds were selected based on their previously reported importance to raspberry aroma [11, 22, 31–33] as well as their representation to various chemical classes including alcohol, aldehyde and ketone, ester, and terpene and tepene alcohol. The concentrations of (in ppb) these volatiles for 2005 are listed in Tables 2 and 3.

There were few differences in volatile concentrations between the transgenic lines and the wild-type 'Meeker'. However, transgenic 2174BO appeared to have higher hexanal and (E)-2-hexenal than the wild-type 'Meeker' and the rest of transgenic lines. Hexanal and (E)-2-hexenal are generated from enzymatic oxidative degradation of fatty acids [26, 34] by lipoxygenase (LOX) and are responsible for the 'green' odor notes and their concentrations are typically related with fruit maturity. However, these differences were not observed with the fruit from Oregon in 2004 or from Washington or Oregon in 2005. Indicating that this variation was likely due to differences in fruit ripeness at harvest rather than genetic based. Similarly, the volatile composition for all the transgenic lines and the wild-type was very similar in Oregon during 2004.

In 2005, some of the volatile compounds had wider range of variations in Washington; however, these variations were not seen in the Oregon grown fruit in either year. None of the variations was consistent from site to sites or year to year. The results suggested that none of the transgenic lines were different from the wild-type. Much greater variation was observed between sites and harvest seasons than between transgenic and wild-type 'Meeker'.

Raspberries grown in Oregon appeared to have higher concentrations of δ-octalactone, δ-decalactone, geraniol and linalool. This trend was observed in both 2004 and 2005, indicating that these compounds are likely to be linked to differences in growing conditions between the locations, particularly the quantity and intensity of sunlight during the growing season.

Overall, the variation observed in °Brix, titratable acidity, organic volatiles was very similar between wild-type 'Meeker' red raspberry and the five transgenic lines evaluated. There was more variation from year-to-year or between sites than there was between wild-type and transgenic lines demonstrating that the fruit from the transgenic lines is indistinguishable from wild-type.

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Tables

Table 1. °Brix, Titratable acidity, original pH and °Brix/TA ratio for wild-type and transgenic raspberries grown in Oregon and Washington during 2004 and 2005.

Location	°Brix	Titratable acidity	pH	°Brix/TA
Wild Type 'Meeker' Lynden, WA 2004	12.9	1.13	3.10	11.36
Transgenic 2171BJ Lynden, WA 2004	12.1	1.11	3.20	10.89
Transgenic 2172AG Lynden, WA 2004	13.6	1.17	3.08	11.61
Transgenic 2174BO Lynden, WA 2004	13.6	1.22	3.16	11.16
Transgenic 2174BS Lynden, WA 2004	13.0	1.16	3.18	11.19
Wild Type 'Meeker' Aurora, OR 2004	13.7	1.24	3.03	11.01
Transgenic 2171BJ Aurora, OR 2004	14.9	0.96	3.01	15.45
Transgenic 2172AG Aurora, OR 2004	13.9	0.95	3.04	14.63
Transgenic 2172BJ Aurora, OR 2004	15.2	1.09	3.02	13.91
Transgenic 2174BO Aurora, OR 2004	14.6	1.11	3.05	13.16
Transgenic 2174BS Aurora, OR 2004	15.9	1.14	3.02	13.99
Wild Type 'Meeker' Lynden, WA 2005	10.8	1.63	2.58	6.63
Transgenic 2171BJ Lynden, WA 2005	10.5	1.52	2.57	6.93
Transgenic 2172AG Lynden, WA 2005	10.4	1.50	2.65	6.88
Transgenic 2172BJ Lynden, WA 2005	10.4	1.43	2.67	7.26
Transgenic 2174BO Lynden, WA 2005	11.0	1.59	2.57	6.90
Transgenic 2174BS Lynden, WA 2005	10.7	1.55	2.62	6.85
Wild Type 'Meeker' Aurora, OR 2005	13.4	1.24	2.82	10.78
Transgenic 2171BJ Aurora, OR 2005	13.7	1.32	2.86	10.32
Transgenic 2172AG Aurora, OR 2005	13.4	1.03	2.90	12.98
Transgenic 2172BJ Aurora, OR 2005	13.7	1.33	2.82	10.27
Transgenic 2174BO Aurora, OR 2005	13.2	1.28	2.82	10.29
Transgenic 2174BS Aurora, OR 2005	13.4	1.35	2.85	9.85

Table 2. Aroma compound concentrations (ppb) for wild-type and transgenic 'Meeker' red raspberries grown in Oregon during 2005.

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS
(Z)-3-Hexenol	249±18.4	204±13.0	218±2.8	189±8.2	210±25.1	192±1.9
4-Isopropylbenzyl alcohol	64±2.3	55±10.0	50±1.8	64±4.4	63±4.4	61±1.3
6-Methyl-5-hepten-2-ol	107±6.3	81±11.6	103±6.7	84±6.5	86±9.6	96±1.2
2-Nonanol	7±0.4	5±0.9	6±0.3	6±0.1	8±0.2	7±0.1
Hexanal	170±16.6	175±31.6	176±6.0	126±7.6	168±15.8	155±5.3
(E)-2-Hexenal	425±30.5	385±210.9	468±0.7	467±11.4	481±26.2	477±11.1
(Z)-3-Hexenyl acetate	11±0.7	4±0.7	5±0.4	6±0.2	6±0.5	7±0.3
Ethyl hexanoate	11±0.7	6±2.4	2±0.1	8±0.1	12±1.6	10±0.6
Methyl nonanoate	1±0.2	1±0.8	1±0.4	1±0.2	1±0.4	1±0.1
2-Heptanone	108±7.5	59±38.8	87±0.9	87±4.8	85±7.4	105±5.8
2-Nonanone	36±2.4	24±2.7	34±1.0	31±1.5	33±1.5	33±2.1
Raspberry ketone	2443±682	2196±532	1672±72	2582±319	1728±743	2746±476
Zingerone	234±44.4	181±21.7	160±10.5	218±20.1	182±37.0	206±35.1
δ-Octalactone	547±24.2	542±63.2	446±12.9	583±13.6	518±52.9	595±4.6
δ-Decalactone	625±21.8	740±275.1	516±4.8	627±10.1	591±24.1	622±6.0
Para cymene	24±2.8	13±10.7	7±7.2	23±1.0	22±1.3	23±2.0
Geraniol	155±5.9	163±13.2	161±1.0	166±8.2	166±10.8	150±6.67
α-Ionone	53±2.6	63±23.1	56±1.2	54±1.6	52±2.0	59±1.2
β-Ionone	73±3.3	84±34.9	66±1.0	70±1.3	72±2.9	74±1.0
Limonene	2±0.4	1±0.5	2±0.2	2±0.1	2±0.2	2±0.4
Linalool	15±0.9	16±0.8	16±1.1	17±1.0	20±1.6	17±1.2
Myrcene	4±5.4	9±2.3	23±0.6	13±6.2	15±12.1	20±5.8
Nerol	27±1.8	21±3.6	25±0.2	25±0.9	27±1.3	25±0.6
α-Phellandrene	49±20.5	44±11.9	61±6.3	58±46.7	45±16.7	59±7.8
α-Pinene	33±3.5	31±5.3	26±0.5	28±1.5	28±3.7	27±1.9
Sabinene	30±4.3	14±0.0	21±2.8	25±1.0	23±1.9	23±3.4
α-Terpinene	25±5.2	13±3.8	15±1.9	20±2.1	17±2.0	19±2.4
γ-Terpinene	25±3.1	13±9.4	14±1.3	23±2.6	6±5.5	18±1.8
α-Terpineol	22±1.3	20±1.2	19±0.5	23±1.0	25±2.1	21±0.9
Terpinen-4-ol	172±7.4	128±36.8	137±1.9	162±9.3	161±10.7	153±6.9
Terpinolene	4±0.5	3±0.5	2±0.2	4±0.4	3±0.3	3±0.3

Table 3. Aroma compound concentrations (ppb) for wild-type and transgenic 'Meeker' red raspberries grown in Washington during 2005.

Compound	Wild type	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS
(Z)-3-Hexenol	149±8.1	141±4.3	168±6.9	152±24.0	153±10.5	188±18.0
4-Isopropylbenzyl alcohol	45±0.6	43±2.0	32±1.8	57±0.8	37±0.6	34±3.3
6-Methyl-5-hepten-2-ol	63±5.3	54±1.6	66±1.3	56±3.2	57±2.6	69±6.7
2-Nonanol	3±0.1	5±0.1	3±0.3	10±0.3	3±0.0	4±0.1
Hexanal	132±3.0	120±5.1	126±15.6	133±15.2	101±8.2	213±13.6
(E)-2-Hexenal	416±21.8	349±3.8	285±14.7	365±15.4	309±14.5	538±45.0
(Z)-3-Hexenyl acetate	6±0.7	5±0.2	7±0.0	5±0.4	5±0.1	5±0.3
Ethyl hexanoate	6±0.4	8±0.3	7±0.5	8±0.3	8±0.4	6±0.2
Methyl nonanoate	1±0.3	1±0.4	ND	1±0.1	ND	ND
2-Heptanone	84±5.9	96±5.2	66±45.7	102±2.3	88±7.7	117±7.1
2-Nonanone	20±2.1	44±1.9	22±0.1	49±3.0	24±0.9	26±1.2
Raspberry ketone	2941±292	2613±1187	1957±1189	3070±471	1738±491	2350±1592
Zingerone	220±12.7	187±47.9	291±98.9	350±29.1	177±27.1	236±102.0
δ-Octalactone	375±14.0	438±22.3	327±19.9	493±12.3	418±3.6	395±40.8
δ-Decalactone	476±24.5	498±5.5	433±8.5	523±9.0	497±5.8	462±29.9
Para cymene	20±1.5	20±1.7	10±1.7	24±1.7	18±1.8	16±1.2
Geraniol	126±12.1	121±7.7	131±1.4	148±7.3	123±7.0	132±8.7
α-Ionone	89±5.2	82±1.5	53±1.3	75±3.4	75±0.1	80±1.6
β-Ionone	94±5.3	94±2.7	72±0.6	96±4.3	88±0.4	90±1.5
Limonene	2±0.2	2±0.1	1±0.1	2±0.2	2±0.2	2±0.2
Linalool	14±1.1	10±0.2	10±0.2	12±0.3	13±0.4	12±1.0
Myrcene	5±1.0	4±0.6	16±7.5	4±1.1	9±6.6	2±1.8
Nerol	21±2.7	20±1.5	21±1.3	29±1.7	20±1.2	20±1.5
α-Phellandrene	57±15.7	51±10.5	33±19.1	72±45.9	59±24.7	54±24.8
α-Pinene	25±1.8	28±0.8	30±3.4	39±2.2	34±3.1	29±0.9
Sabinene	22±2.8	24±2.1	15±7.4	37±0.9	23±1.3	23±2.3
α-Terpinene	23±1.8	22±0.4	10±4.9	40±10.1	20±0.8	21±2.1
γ-Terpinene	24±2.3	21±0.9	6±6.7	49±4.0	20±1.6	24±0.5
α-Terpineol	18±1.4	16±0.4	14±0.1	22±0.7	19±0.8	17±1.6
Terpinen-4-ol	135±14.2	145±6.8	139±2.4	214±8.9	138±6.6	158±12.0
Terpinolene	4±0.4	4±0.1	3±0.1	8±0.7	4±0.3	4±0.2